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MYOGLOBIN IN CARBON MONOXIDE INTOXICATION OF THE BODY

[Mioglobin pri otruyenni organizmu okisom vugletsyu]

by

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It was found that the relation between the content of HbCO and MbCO is asymptotic in rats poisoned with mixtures of air and carbon monoxide in various concentrations (0.01-0.10%) in an equilibrium state. With pCO a rise in inspired air the quantity of CO-compounds increases: HbCO ranges at an average from 1 to 55%, MbCO--from 0 to 27%. At higher concentrations of CO the quantity of HbCO and MbCO is preserved at the maximum level.

The distribution of carbon monoxide in the body can be analyzed in terms of its relative affinity for various blood proteins, its quantity, and by the location of their dispersal. CO is bonded predominantly in the blood hemoglobin and muscular myoglobin. In a state of equilibrium the basic distributive indices of CO in the body are factors in the

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Golden equation, i.e., muscular pCO, pO_2 content of Hb and Mb. Isolated data are cited in the literature relating to the relative magnitudes of CO bonding with Hb and Mb [1-4]. Earlier [5] we discussed the quantitative laws of HbCO and MbCO formation in the body of rats as a function of pCO in inspired air (0.15-76 mm Hg). In that study the intoxication time varied (5-60 min.) and it was theoretically established that no state of equilibrium was achieved in CO between inspired breaths, the blood and the muscles.

We are now intent on establishing the laws of formation of CO compounds of those pigments in a state of diffusive equilibrium with the inspiration of CO-air mixtures having some content of CO (0.076-0.76 mm Hg).

Methods

Forty-eight white laboratory rats weighing 200-400 g were used as test animals. The intoxication system was composed of a series-connected gasometer, a flask containing a 10% NaOH solution to remove traces of CO_2, and H_2O to moisten the CO-air mixture, a 7 liter desiccator in which the animals were placed, and flasks containing water as a barometric seal. The experiment lasted for four hours.

Optimum intoxication conditions--a gas mixture flow rate and CO_2 accumulation--was calculated by us mathematically. The experimental concentration of CO in the desiccator, in the range of 0.01-0.10%, was maintained for 10 min. with a CO-air mixture flow rate in this period of 0.5 liters/minute. It had been calculated that the flow rate of gas mixtures containing 0.01-0.50% of CO for 10 minutes in the apparatus established

a state of equilibrium. This CO concentration was maintained constant until the end of the experiment; the subsequent gas flow rate was 0.2 l/min. The amount of inspired carbon dioxide (in ml) increased during the experiment and achieved a state of equilibrium after 3 hrs; by 4 hrs it was 2.68%. This concentration of CO_2 was taken as the maximum allowable concentration (MAC).

The concentration of CO and CO_2 in the desiccator, as a function of the gas mixture flow rate and time, were calculated using the following formula:

$$C = (C_0 - \frac{Q}{V})\, e^{-\frac{vt}{V}} + \frac{Q}{V},$$

where C--the concentration of CO (or CO_2) in the desiccator, C_0--CO concentration (or CO_2) in desiccator at time zero; Q--accumulation of CO (or CO_2) in desiccator after time t; v--flow rate of gas mixture through desiccator, V--volume of desiccator.

In one experiment, 51 liters of a carbon monoxide and air mixture were used. After exposure for four hours the rats rapidly became violent and strangely pallid. The muscles were treated by the method described [4] earlier to retain the myoglobin solutions. The Hb solutions were maintained by diluting defibrinated blood with a phosphate buffer at pH 7.4. To restore the CO-compound solvents, the solutions were prepared in the dark and at a cool temperature. The percentage content of CO compounds in the Mb and Hb solutions was analyzed by spectrophotometry [5, 6].

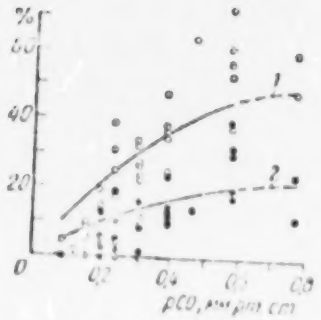

Fig. 1. Relationship of HbCO (1) and MbCO (2) in rat organisms as a function of the value pCO in inspired air.

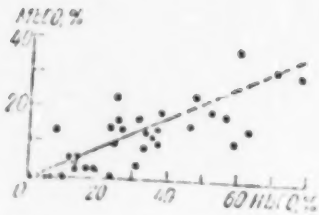

Fig. 2. Relationship of MbCO Formation in Muscles as a function of HbCO content in the blood.

Duration of poisoning: 4 hours. Some experimental data concerning percentage concentration of CO compounds of the pigments for the value of pCO were equated with the formula of asymptotic increase [7]: $HbCO = 59.06 - 59.06 \cdot 10^{-0.85X}$; $MbCO = 28.29 - 28.29 \cdot 10^{-0.99X}$.

Test Findings

Based on the data curves of HbCO and MbCO dissociation were constructed which express the relationship of the percentage mixture of saturated HbCO and MbCO in the animal organisms as a function of partial CO pressure in the inspired air (Fig. 1).

Individual variations are noted in the amounts of CO-compounds. In order to balance out the series of experimental data, we used mathematical processing which was employed to compute the formula of asymptotic increase [7]: $Y = A - D \cdot 10^{-kx}$.

With an increase in the value of pCO in the inspired air from 0.076 to 0.570 mm Hg column, the amount of CO-compounds gradually rises and attains the maximum: HbCO of the blood--an average of 1 to 55%; MbCO of the muscles--from 0 to 27%. The amount of CO compounds in the pig-

ments maintains the maximum value with further increase in CO concentration in inspired air. The rats survived these test conditions for the four hours of the experiment.

As data obtained will confirm, with an increase in the HbCO content in the blood, much CO shifts from the blood to the muscles; this may be attributed to the high pCO gradient. There is a linear relationship between the formation of MbCO in the rats' muscles and the HbCO in the blood (Fig. 2). The formation of MbCO is easily observed in the muscles when the HbCO content in the blood is over 10%. We were unable to preclude the possibility that small amounts of MbCO decomposed during extraction and purification. The relative distribution of CO between the Mb and Hb (i.e., the percentage relationships between the MbCO and HbCO contents) for our experimental conditions remained a constant 0.45 (Fig. 3). This relationship is not a function of the HbCO quantity.

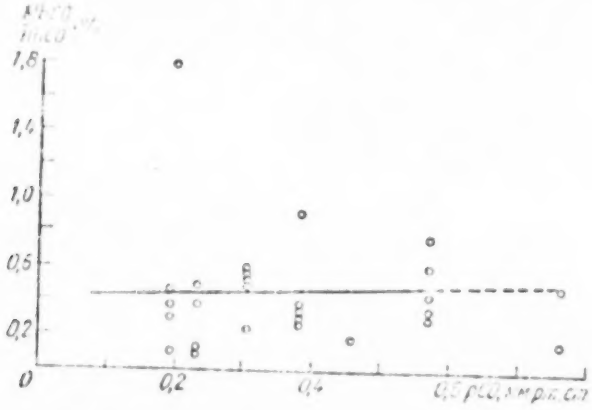

Fig. 3. Relative distribution of CO among Hb and Mb in the body of rats, a function of the pCO value in inspired air.

Discussion of Data Obtained

The distribution of CO between the blood and tissues is primarily the result of the following factors: blood supply of different tissues; ability of CO to diffus through the membranes into tissues; their ability to absorb CO, combine with it or dissolve it.

In our research we came to the conclusion that myoglobin is the main CO acceptor in the body aside from Hb. In order to quantitatively estimate the distribution of CO between the blood Hb and muscle Mb as a function of the CO content in inspired air, it was necessary to select a length of experimentation which would ensure the state of diffusive equilibrium where the amount of CO in expired air would be equal to its quantity in inspired air.

We decided upon a four-hour period of intoxication, which enables us to state that at this exposure, CO migrates from the vessels into the muscles and is in a state of equilibrium between the blood and muscles. In the state of equilibrium the distribution of CO in the body may be analyzed by the factors of the Golden equation:

$$\frac{(HbCO)}{(HbO_2)} = K\frac{pCO}{pO_2}; \qquad (MbCO) = K\frac{pCO}{pO_2}.$$

To check the state of equilibrium with the Golden equation, a value was found analogous to the constants of equilibrium K of Hb and Mb with O_2 and CO <u>in vivo</u> (cf. table). To compute K_{Hb}, pO_2 and pCO were taken as equal to the partial pressures of these gases in inspired air; in calculating K_{Mb}, pO_2 in the muscles was 10 mm Hg and pCO--1/3 pCO in the inspired air [8]. Partial pressure of CO in tissues, in conformity with the law of action of mass, is proportional to the HbCO contents:

$$pCO = \frac{(HbCO)\,pO_2}{(HbO_2)\,K}.$$

In the table, the values of K' are similar to those of K established for Hb and Mb of a rat *in vitro* (K_{Hb} = 159 ± 44, K_{Mb} = 34 ± 12 [9]), which would prove the attainment of a state of equilibrium of CO between the inspired air, blood and muscles. The theoretical values of HbCO and MbCO were also calculated for the experimental content of CO with the Golden equation. The value of their theoretical relationship is equal to unity.

Table 1.

Relative percentage of Hb and Mb with O_2 and CO in Rat Organisms

pCO, мм рт. ст.	a						b						
0,076	2000,00	10,26	89,81	0,113	2,63	7,36	0,025	100,00	4,36	95,64	0,045	18,40	7,83
0,111	1333,33	14,70	85,30	0,172	2,53	10,65	0,038	100,00	6,25	93,71	0,067	17,63	11,44
0,152	1000,00	18,70	81,30	0,230	2,50	13,52	0,051	100,00	8,06	91,94	0,088	17,26	14,78
0,190	800,00	22,33	77,67	0,287	2,52	16,75	0,063	100,00	9,67	90,33	0,107	16,98	17,64
0,229	666,67	26,11	73,89	0,345	2,60	19,79	0,076	100,00	11,19	88,81	0,126	16,58	20,53
0,301	500,00	31,31	68,69	0,439	2,60	24,13	0,101	100,00	13,84	86,16	0,161	15,94	25,59
0,380	400,00	36,20	63,80	0,567	2,69	28,44	0,127	100,00	16,01	83,99	0,191	15,01	31,16
0,456	333,33	40,16	59,84	0,672	2,74	32,39	0,152	100,00	17,91	82,09	0,219	14,41	34,67
0,570	266,67	44,84	55,16	0,813	2,80	37,55	0,190	100,00	20,25	79,75	0,254	13,37	39,25

Key: a. blood b. muscles c. theoretical

[Translator's note: Commas in the tabulated figures are equivalent to decimal points.]

Note: K_{Hb} = 159 ± 41 (in vitro), pO_2 = 152 мм рт. ст.; $HbCO,\ \%\ (теор.) = \frac{100\,K\,pCO}{K\,pCO + pO_2}$; $K_{Hb} = \frac{pO_2\,HbCO}{pCO\,HbO_2}$; K_{Mb} = 31 ± 12 (in vitro); pO_2 = 10 мм рт. ст.; pCO = 1,3 pCO in inspired air; $MbCO,\ \%\ (теор) = \frac{100\,K\,pCO}{K\,pCO + pO_2}$; $K_{Mb} = \frac{pO_2\,MbCO}{pCO\,MbO_2}$.

But we were unable to detect complete saturation of myoglobin by CO in the body.

The migration of CO from the blood Hb to the muscular Mb is a theoretically complex function, governed by many factors such as: circulatory mixing of CO; capillary perfusion of skeletal muscles and the myocardium; by the thickness of capillaries in the skeletal muscles and myocardium; by the diffusibility to CO of the entire membrane between the Hb and Mb; by the pCO gradient between the Hb and Mb; by the pO_2 around Hb and Mb; by the rate of reaction of CO and O_2 with Hb and Mb. The process which defines the migration of CO is the perfusion of striated muscles [1].

The main physiologic function of Mb is as a reservoir of oxygen for the muscular mitochondria. Under the influence of Mb the requirement for glycolysis diminishes under normal conditions and the efficiency of metabolism in the muscles is improved thereby. Moreover, as has recently been found, Mb evokes acceleration of O_2 diffusion into the muscles by means of reciprocal compounding with it and also by translational diffusion of MbO_2 molecules [10]. Though the affinity constant of Mb for CO and oxygen is less than the constant of affinity of Hb for such gases, it is ample to form MbCO in the organism upon the intake of CO. In view of the various sources of CO for the body (endogenic formation and exogenic intake [11]), in healthy individuals the HbCO level may range from 3 to 20%, which elicits the formation of MbCO in the muscles and myocardium.

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